(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
4 March 2010 (04.03.2010)

PCT

(10) International Publication Number
WO 2010/025126 A1

(51) International Patent Classification:
A61K 38/46 (2006.01) *A61K 9/46* (2006.01)
A61K 38/47 (2006.01) *A61P 1/18* (2006.01)
A61K 38/48 (2006.01)

(21) International Application Number:
PCT/US2009/054866

(22) International Filing Date:
25 August 2009 (25.08.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/091,773 26 August 2008 (26.08.2008) US

(71) Applicant *(for all designated States except US)*: **CYSTIC FIBROSIS FOUNDATION THERAPEUTICS, INC.** [US/US]; 6931 Arlington Road, Bethesda, MD 20814 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PECHENOV, Sergey, Y.** [RU/US]; 4409 Merchant Square Place, Landsdale, PA 19446 (US). **BASU, Sujit, Kumar** [IN/US]; 38 Lovett Road, Newton, MA 02459 (US). **MISHRA, Shubhang** [US/US]; 30 Wildwood Drive, Southborough, MA 01772 (US).

(74) Agent: **MCDONAELL, Leslie, A.**; Finnegan, Henderson, Farabow, Garrett & Dunner, LLP, 901 New York Avenue, NW, Washington, DC 20001-4413 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2010/025126 A1

(54) **Title**: RAPIDLY DISINTEGRATING TABLETS COMPRISING LIPASE, AMYLASE, AND PROTEASE

(57) **Abstract**: The present invention relates to orally administrable formulations of proteins, including their amorphous and crystalline phases, and cross-linked forms thereof. In particuler, the formulations comprise a mixture of lipase, protease and amylase together with an effervescent agent. The invention further relates to methods of administration of these formulations, including their preparation and conditions of use. More specifically, the invention relates to methods of preparing and administering formulations that are advantageous for use with pediatric populations and patients with deglutition-related disorders such as those frequently seen in disabled and geriatric populations, and patients with esophageal occlusions or autonomic complications (i.e., from Parkinson's disease, Multiple Sclerosis, Lou Gehrig's disease, etc.).

RAPIDLY DISINTEGRATING TABLETS COMPRISING LIPASE, AMYLASE, AND PROTEASE

[001] The present invention relates to orally administrable formulations of proteins, including their amorphous and crystalline phases, and cross-linked forms thereof. The invention further relates to methods of preparing these formulations, as well as methods of administration and conditions of use.

[002] Food digestion and nutrient absorption occur in the small intestine. There, ingested food is broken down by digestive enzymes for ready absorption. Most digestive enzymes are secreted by the pancreas and arrive in the small intestine through the pancreatic duct.

[003] The pancreas affects a variety of exocrine and endocrine actions required for proper digestion, nutrition and metabolism. Pancreatic exocrine activities include the secretion of proteins that function as enzymes in the small intestine to catalyze the hydrolysis of fat into glycerol and fatty acids, protein into peptides and amino acids and carbohydrates into dextrins, disaccharides and monosaccharides, such as glucose. Exocrine pancreatic insufficiency (hereinafter "pancreatic insufficiency") results from a reduction in pancreatic function and can be caused by a number of clinical disorders. For example, pancreatic insufficiency is associated with cystic fibrosis, chronic pancreatitis, acute pancreatitis, pancreatic cancer and Shwachmann-Diamond Syndrome (E. P. DiMagno et al., in The Pancreas: Biology, Pathobiology and Disease, 2d Ed., V. Liang et al., eds., pp. 665-701 (1993)).

[004] In patients afflicted with pancreatic insufficiency, the pancreas fails to produce and/or secrete sufficient amounts of digestive enzymes to support normal digestive processes, including digestion of fats, proteins and carbohydrates.

As a result, those patients suffer from malabsorption of nutrients. Clinical manifestations of pancreatic insufficiency include abdominal cramping, bloating, diarrhea, steatorrhea, nausea and weight loss.

[005] Pancreatic insufficiency is present in 89% of patients suffering from cystic fibrosis (D. Borowitz et al., "Use of Fecal Elastase-1 to Identify Misclassification of Functional Pancreatic Status in Patients with Cystic Fibrosis", J. Pediatr., 145, pp. 322-326 (2004)). Cystic fibrosis is an autosomal recessive genetic disorder that primarily affects the gastrointestinal and respiratory systems (S. M. Rowe et al., "Mechanisms of Disease: Cystic Fibrosis", N. Engl. J. Med., 352, pp. 1992-2001 (1995)). Abnormal amounts and viscosity of mucus produced in cystic fibrosis patients impede the secretion of sufficient amounts of pancreatic enzymes. The decreased volume of pancreatic secretions leads to inspissation within the pancreatic ducts, preventing egress of enzymes and bicarbonate into the duodenum. As a result, cystic fibrosis patients with pancreatic insufficiency suffer from impaired digestion and experience significant malabsorption of fat and protein. For example, such patients typically absorb less than 60% of dietary fat (M. Kraisinger et al., "Clinical Pharmacology of Pancreatic Enzymes in Patients with Cystic Fibrosis and *in vitro* Performance of Microencapsulated Formulations", J. Clin. Pharmacol., 34, pp. 158-166 (1994)). If left untreated, maldigestion and malabsorption in cystic fibrosis patients lead to malnutrition, inability to gain or maintain weight and decreased growth, as well as worsening of chronic suppurative lung disease (K. Gaskin et al., "Improved Respiratory Prognosis in CF Patients with Normal Fat Absorption", J. Pediatr., 100, pp. 857-862 (1982); J. M. Littlewood et al., "Control of Malabsorption in Cystic Fibrosis", Paediatr. Drugs, 2, pp. 205-222 (2000)).

[006] Distal intestinal obstruction syndrome (DIOS) also causes pancreatic insufficiency. This condition is characterized by the accumulation of viscous mucous and fecal material in the terminal ileum, caecum and ascending colon. The syndrome is found to occur after the neonatal period and ranges from a few mild to several excrutiatingly painful episodes, increasing progressively from adolescence to adulthood. Contributing factors may include fat malabsorption (Anderson et al., J. Pediatr Gastroenterol Nutr 11:356-60 (1990)), abnormally thick mucin secretions, low duodenal pH, low dietary fibre intake, abnormal water and electrolyte transport and the use of anticholinergic drugs (Eggermont, Eur. J. Gastroenterol Hepatol 8:731-8 (1996)).

[007] Mild or moderate episodes of DIOS result in intermittent abdominal discomfort and occasional bloating. Chronic onset of the disease, however, results in severe malnourishment if left untreated, followed by decreased energy and a cascade of events leading to anorexia, advanced pulmonary disease and eventually, death.

[008] Patients with Shwachman-Diamond syndrome, a rare congenital disorder, also suffer from pancreatic insufficiency. Pancreatic insufficiency is also present in some patients suffering from type I and type II diabetes. The standard therapy for pancreatic insufficiency is pancreatic enzyme replacement therapy. This therapy is primarily orally-administered porcine pancrelipase, containing a mixture of lipases, trypsin, chymotrypsin, elastase and amylases. The lipase, protease and amylase components of porcine pancreatic supplements are typically present in a 1:3.5:3.5 ratio.

[009] Mild or moderate episodes of DIOS can often be treated medically with pancreatic enzyme replacement therapy and laxatives. However, severe cases

of both DIOS and cystic fibrosis may also surgical interventions to enable enteral nourishment and gastronomic administration of said medications via feeding tubes. Short-term benefits of feeding tubes include immediate weight gain and increased energy. Long-term gains include an increase in body fat, lean muscle mass, improved strength, a stronger immune system, less weight loss during pulmonary infections, a greater sense of control over body weight and numerous other humoral benefits. However, despite the obvious benefits offered by feeding tubes, gastronomic administration of solid oral dosage medicines is complicated by a number of preparative and administrative challenges that may render the active pharmaceutical ingredients ineffective. A summary of the advantages and potential complications of enteral feeding is provided in Pearce and Duncan, Postgrad. Med. J. 78:198-204 (2002).

[010] Other disadvantages of present methods of enteral nutrition include surgical and post-operative complications, tube displacement and stabilization, incorrect tube size and type, leakage, risk of infection, skin irritation, patient discomfort, and frequent occlusions that form in the gastric tube due to formula and medicinal precipitations, inadequate flushing, and drug-nutrient interactions. This aspect becomes particularly significant for cystic fibrosis patients who require their oral medications to be administered enterally, including pancreatic enzyme-replacement therapies. That is to say, despite the availability of various rapidly-disintegrating tablets and formulations such as those mentioned above, delivery of these formulations through feeding tubes to the intended target site presents serious challenges to be overcome.

[011] Furthermore, because most feeding tubes are made of polyurethane or silicon, (i.e.: The Corflow PEG® by Merk Biomaterials, Inc.), conditions of use,

sample preparative techniques, and methods of selecting appropriate inert disintegration media, present important challenges to prevent or mitigate unfavorable interactions between the mixture and the tubes. Interestingly, Bourgault et al., Nutr. Clin. Pract. 18:398 (2003) suggests that prophylactic administration of pancreatic enzymes may reduce feeding tube occlusions.

[012] Pancreatic enzyme supplements are normally administered orally with meals. As these supplements pass through the low pH environment of the stomach, their enzyme activity diminishes rapidly. As a result, large quantities of enzyme concentrate (sometimes as many as 15 capsules or tablets per meal) have been required to ensure that sufficient active enzyme is present in the proximal intestine to relieve pancreatic insufficiency.

[013] Because protease and lipase can become irreversibly inactivated in the stomach's acidic environment, enteric-coating technologies have been applied to pancrelipase products, to enclose enzymes in microbeads or otherwise treat them with a protective enteric coating. While such enteric-coatings improved the product profile, large quantities of supplements were still required to yield therapeutic benefit (J. H. Meyer, in Pancreatic Enzymes in Health and Disease, P. G. Lankisch, ed., pp. 71-88 (1991)). A high-strength pancrelipase product line (Ultrase™) was introduced, with the goal of reducing the quantities of tablets or capsules necessary to treat pancreatic insufficiency. However, in 1991 the United States Cystic Fibrosis Foundation, in conjunction with the FDA, reported multiple cases of fibrosing colonopathy in children with cystic fibrosis taking such high-strength products (S. C. Fitzsimmons et al., "High-Dose Pancreatic-Enzyme Supplements and Fibrosing Colonopathy in Children with Cystic Fibrosis", N. Engl.

J. Med., 336, pp. 1283-1289 (1997)). In these patients, colonic fibrosis caused strictures that often required surgery and, in some cases, colectomy.

[014] As a means toward reducing daily doses of pancreatic enzymes, the FDA removed the high strength products (defined as greater than 2,500 USP units per kg body weight) from the market (D. S. Borowitz et al., "Use of Pancreatic Enzyme Supplements for Patients with Cystic Fibrosis in the Context of Fibrosing Colonopathy", J. Pediatr., 127, pp. 681-684 (1995)). In addition, the United States Cystic Fibrosis Foundation, jointly with the FDA, recommended a detailed examination of the complex nature of porcine enzyme extracts (Id.). The Consensus Panel also recommended investigation of alternative, acid-stable lipases.

[015] Whether or not a given pancreatic enzyme supplement is enterically-coated, the bioavailability of such supplements varies widely, due to differentials in acidification of the intestine among patients. As a result, many patients take pH altering drugs, such as histamine-2 (H.sub.2) receptor blockers and proton pump inhibitors (PPI), to improve the clinical efficacy of the enzyme supplements (P. G. Lankish, "Enzyme Treatment of Exocrine Pancreatic Insufficiency in Chronic Pancreatitis', Digestion, 54 (Supp. 2), pp. 21-29 (1993); D. Y. Graham, "Pancreatic Enzyme Replacement: the Effect of Antacids or Cimetidine", Dig. Dis. Sci., 27, pp. 485-490 (1982); J. H. Saunders et al., "Inhibition of Gastric Secretion in Treatment of Pancreatic Insufficiency", Br. Med. J., 1, pp. 418-419 (1977); H. G. Heijerman et al., "Omeprazole Enhances the Efficacy of Pancreatin (Pancrease) in Cystic Fibrosis", Ann. Inter. Med., 114, pp. 200-201 (1991); M. J. Bruno et al., "Comparative Effects of Adjuvant Cimetidine and

Omprazole during Pancreatic Enzyme Replacement Therapy", Dig. Dis. Sci., 39, pp. 988-992 (1994)).

[016] Variability in terms of potency and pharmaceutical properties and lack of stability have also been identified as important factors contributing to a poor response of some patients to conventional pancreatic enzyme supplements (C. L. Chase et al., "Enzyme Content and Acid Stability of Enteric-Coated Pancreatic Enzyme Products *in vitro*", Pancreas, 30, pp. 180-183 (2005); D. S. Borowitz et al., J. Pediatr., 127, supra; C. J. Powell et al., "Colonic Toxicity from Pancreatins: a Contemporary Safety Issue", Lancet, 353, pp. 911-915 (1999); E. Lebenthal et al., "Enzyme Therapy for Pancreatic Insufficiency: Present Status and Future Needs", Pancreas, 9, pp. 1-12 (1994); P. Regan et al., "Comparative Effects of Antacids, Cimetidine and Enteric Coating on the Therapeutic Response to Oral Enzymes in Severe Pancreatic Insufficiency", N. Eng. J. Med., 297, pp. 854-858 (1977)). These include batch-to-batch variation in enzyme activity, susceptibility to loss of activity over time by exposure to sunlight, heat or humidity and a poorly defined profile of adverse reactions (D. S. Borowitz et al., J. Pediatr., 127, supra). Other factors that complicate pancreatic insufficiency therapy include destruction of the replacement enzymes by gastric juice and/or intraluminal proteases, asynchronous gastric emptying of enzyme supplement and meal nutrients, and delayed liberation of enzyme from enteric-coated preparations (P. G. Lankish, Digestion, 54 supra; P. Regan et al., N. Eng. J. Med., 297, supra).

[017] Due to the problems of potency, stability and bioavailability characterizing conventional pancreatic enzyme supplements, the use of microbially-derived enzymes as alternatives to porcine-derived enzymes has been proposed. For example, U.S. Pat. No. 6,051,220 describes compositions comprising one or

more acid stable lipases and one or more acid stable amylases, both preferably of fungal origin. United States patent application 2004/0057944 describes compositions comprising *Rhizopus delemar* lipase, *Aspergillus melleus* protease and *Aspergillus oryzae* amylase. United States patent application 2001/0046493 describes compositions comprising crosslinked crystalline bacterial lipase, together with a fungal or plant protease and a fungal or bacterial amylase.

[018] However, despite these significant advances, the management of pancreatic insufficiency in certain populations has been unfortunately hindered by the reluctance and/or inability to swallow the above medication in traditional solid oral dosage forms, such as capsules, pills or tablets. This is particularly true, for example, in the disabled, pediatric and geriatric populations, patients with esophageal occlusion, and those with autonomic complications (i.e., from Parkinson's disease, Multiple Sclerosis, Lou Gehrig's disease, etc.). Accordingly, efforts have been made in the past to formulate medications in forms which are easier to swallow. Examples of such efforts include coatings of tablets and pills; fast disintegrating tablets for sublingual or subbucchal administration.

[019] Tablets and capsules are the preferred dosage forms of choice for oral administration, availing a number of different shapes and sizes for oral use. These range from 1.0 to 2.5 cm in height or less, and 0.25 to 1.5 cm in diameter or less, and can usually be self-administered by patients without the need for complex apparati, such as those used in parenteral, intranasal, ophthalmic or other administration systems. However, while most of the capsules and tablets available today are relatively easy to swallow, sometimes even the smallest ones can pose significant challenges for disabled, pediatric and geriatric patient populations and those with esophageal or gastro-intestinal disorders. Administration of the drug in

these situations entails either crushing the tablets or opening the capsules, and resuspending the contents in suitable liquid or semi-liquid mediums (such as baby food, apple sauce) for oral delivery.

[020] Accordingly, a need still exists for alternate formulations of pancreatic enzymes for delivery to disabled, pediatric and geriatric patient populations. Preferably, these formulations will rapidly disintegrate in a variety of solutions while maintaining the protein therapeutics in an active form during disintegration and administration.

[021] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DESCRIPTION OF THE EMBODIMENTS

[022] The present invention is the first preparation of a protein therapeutic in an formulation useful for disabled, pediatric and geriatric patient populations and those with esophageal or gastro-intestinal disorders. The incorporation of protein therapeutics into a rapidly disintegrating formulation requires the manipulation of a significant number of parameters. While this approach may appear relatively simple, it is highly dependent on the chemical and physical compatibility of the therapeutic and the other components of the formulation, and, optionally, the disintegration media. Arbitrarily chosen excipients and disintegration media that are marginally compatible or not compatible at all with the therapeutic can result in an irrecoverable loss of therapeutic activity.

[023] New findings further indicate that this risk is further compounded in the oral administration of protein-based therapeutics such as the three-pancreatic-enzyme replacement therapy, liprotamase, described in U.S. Publication No.

2006/0121017. Since each of the proteins and protein crystals in this gastrointestinal product have distinct pH-optimal and solubility ranges for therapeutic activity, the choice of suitable formulation components becomes even more significant. Thus, in addition to the fundamental design of formulations, appropriate techniques of selecting suitable disintegration media for liquid and semi-solid administrations, solid oral dosage forms, and preparative methods thereof, are particularly eminent and paramount.

[024] Proposed enzyme replacement therapy may be further complicated in critically ill patients suffering from DIOS who require enteral feedings or gastronomic administration of their medications in liquid or semi-solid media. Thus, the present invention provides materials and methods for disintegration media and preparative techniques that are effective for such administration, maintaining the stability of the dispersed protein therapeutic during and after administration, while also preventing or minimizing unfavorable chemical or physical interaction with the gastric tubes and components thereof.

[025] For example, if a rapidly-disintegrating formulation of liprotamase is dissolved and/or dispersed in water for enteral administration, although the pH of the mixture would be lower than water, the mixture would remain buffered so as to maintain the therapeutic activity of the enzymes, and prevent or minimize potentially unfavorable interactions. In related embodiments of the present invention, the methods and materials of the present invention are advantageous in reconditioning and maintaining the patency of gastric tubes and buttons that may become occluded due to formula and medicinal precipitation, inadequate flushing and drug-nutrient interactions.

[026] The present invention relates to stable formulations of pharmaceutically active proteins, including their amorphous and crystalline phases, and cross-linked forms thereof. More specifically, it addresses the aforementioned need by providing materials and methods for rapidly disintegrating tablets and other dosage forms which enable controlled disintegration of the said forms in suitable disintegration and disintegration media *ex vivo*, followed by oral administrations of the resulting combination comprising the disintegrated and dispersed products and media.

[027] In preferred embodiments, the compositions according to the present invention include those comprising crosslinked microbial lipase crystals, a microbial protease and a microbial amylase, such as those described in U.S. Publication No. 2006/0121017.

[028] In particular embodiments, the invention relates to a composition comprising from about 4.0 to about 50.0 weight percent of a mixture of lipase, protease, and amylase; from about 30 to about 90 weight percent excipients; and from about 2.0 to about 30.0 weight percent effervescent agent, wherein the composition is capable of rapidly disintegrating in the oral cavity. In some embodiments, the composition is a tablet. In some embodiments, the lipase, the protease and the amylase of liprotamase are each recombinantly produced. In some embodiments, the lipase is crystallized. In some embodiments, the crystallized lipase is cross-linked. In some embodiments, the lipase crystals remain undissolved at gastric pH levels of 2.8 to 4.0.

[029] In particular embodiments, the composition disintegrates in water in about 30 seconds to 2 minutes. In some embodiments, the lipase, amylase, and protease maintain about 50-100% of their initial biological activity after

disintegration in water. In particular embodiments, the lipase, amylase, and protease maintain at least about 25%, 35%, 45%, 55%, 65%, 75%, 85%, 95%, or 100% of their initial biological activity after disintegration in water.

[030] In particular embodiments, the effervescent agent is an effervescent couple. In some embodiments, the effervescent couple is sodium bicarbonate and citric acid. In some embodiments, the effervescent agent is sodium bicarbonate.

[031] In particular embodiments, the invention relates to a method of providing a composition of lipase, protease, and amylase to a patient in need of same comprising combining the rapidly disintegrating dosage form of claim 1 with a suitable disintegration media. In some embodiments, the disintegration media is cranberry apple juice, water, or apple juice. In particular embodiments, the invention relates to a method for reducing gastric tube occlusions, the method comprising treating a gastric tube with the compositions of the invention.

[032] In particular embodiments, the invention relates to the use of the compositions of the invention for the preparation of a medicament for the treatment of pancreatic insufficiency. In other embodiments, the invention relates to the use of the compositions of the invention for the prevention of gastric tube occlusions.

[033] Lipase and protease are preferably present in a ratio of from about 1.0:0.5 to about 1.0:1.5 USP units of enzyme activity, more preferably about 1.0:0.7 to 1.0:1.0 USP units. Lipase and amylase are preferably present in a ratio of from about 1.0:0.05 to 1.0:0.3 USP units of enzyme activity, more preferably from about 1.0:0.10 to about 1.0:0.20 USP units. In preferred embodiments, the lipase is a bacterial lipase and the protease and amylase are of fungal origin. Most preferably, the composition comprises *Burkholderia* lipase crystals crosslinked with bis(sulfosuccini-midyl) suberate ("BS^3") crosslinker, *Aspergillus melleus* protease

crystals and soluble *Aspergillus oryzae* amylase; in a ratio of about 1.0:1.0:0.15 USP units of enzyme activity; or about 1.0:0.77:0.12 USP units of enzyme activity. The active ingredients may be formulated together with one or more excipients.

[034] The crosslinking of the lipase component of the compositions of this invention provides added stability at pH extremes and protection under proteolysis, while the protease and amylase components maintain maximum solubility for effective dissolution. More particularly, the crystallization and cross-linking of the lipase component helps provide a composition with enhanced enzyme activity at lower dosages. The crystal form of the protease also helps to provide enhanced enzyme stability, purity and potency.

[035] In alternate embodiments of the present invention, the lipase may be in any stabilized form, and either or both of the protease and amylase components of the compositions may be in crystal, amorphous or semi-crystalline form. Alternatively, either or both may be in lyophilized form. And, regardless of their form, either or both may be crosslinked.

[036] The lipase component of the compositions of the present invention is preferably a microbial lipase. More preferably, the lipase is bacterial, rather than fungal or of plant origin. The lipase is preferably one that is stable in an acidic pH environment and/or that is resistant to proteolytic degradation. The lipase may also be employed in a form that renders enhances its stability to acidic pH and/or its resistance to proteolytic degradation. To that end, the lipase is preferably in the form of crosslinked crystals. In particular embodiments, the lipase is of *Pseudomonas* or *Burkholderia* origin. Any of the above-described lipases may be used to form a crosslinked lipase crystal component of the compositions of the present invention.

[037] The protease component of the compositions of the present invention is a microbial protease. Preferably, the protease is of fungal, rather than bacterial or plant origin. More preferably, the protease is an *Aspergillus* protease. Most preferably, the protease is *Aspergillus melleus* protease. According to a preferred embodiment, the protease component of the compositions of the present invention is in crystallized, non-crosslinked form.

[038] The amylase component of the compositions of the present invention is a microbial amylase. Preferably, the amylase is of fungal, rather than bacterial or plant origin. More preferably, the amylase is an *Aspergillus* amylase. Most preferably, the amylase is *Aspergillus oryzae*. According to a preferred embodiment, the amylase component of the compositions of the present invention is in amorphous form. Alternatively, the amylase component of the compositions of this invention may be in crystalline forms, including crosslinked and non-crystalline forms, or coated, or encapsulated or otherwise formulated so that it retains its activity after oral administration.

[039] If desired, a taste masking substances, or taste modifiers such as flavoring components or sweeteners can be included in the compositions of the present invention, wherein the said flavors may be, but not limited to, cherry-berry, bubble-gum.

[040] One aspect of the invention comprises one or more formulations with specific properties that enable controlled disintegrations of the aforementioned solid oral dosage forms. In a similar aspect, the said formulations may be pretreated prior to disintegration or administration to delay or accelerate the onset of therapeutic activity

[041] It is well known that the solution or liquid state stability of pharmaceutical agents, especially proteins are generally less compared to the solid state stability of the pharmaceutical agents. This is due to greater mobility of the pharmaceutical agents and increased possibility of their interactions with each other, with the excipients in the formulation and with the container closure system. This is especially true for enzymes, and is particularly significant for a variety of catalytic enzymes (e.g., peptidases and proteases, hydrolases, etc.), which could catalyze other peptides and proteins that are either the therapeutic or excipients in the formulation. In such situations, it is advantageous to formulate the therapeutics and excipients into powder in sachet or fast disintegrating tablets for the disintegration and delivery to leverage the improved solid state stability of the therapeutic combination. For example, because one of the therapeutic proteins in liprotamase is protease and the other two therapeutics are also proteins, the latter can themselves become susceptible to proteolytic degradation by the protease component in the formulation. Moreover, the protease itself becomes subject to autocatalysis when in the solution state.

[042] The duration of stability post disintegration in a media for administration prior to dose administration also need to be established. A variety of factors needs to be considered for the safe and efficacious administration. These include, but are not limited to, pH-solubility profile; pH-activity profile; pH-stability profile; solubility-activity profile; and proteolytic stability profile.

[043] In preferred embodiments of the present invention, the compositions comprise rapidly disintegrating tablets (RDTs) comprising active ingredients, such as those described above for liprotamase, i.e., a combination of lipase, such as a cross-linked crystallized lipase, protease and amylase. The RDTs disperse quickly

in liquids, or in the mouth without chewing or the need for water, making it easier for patients to take the medication. A variety of commercially available fast-dissolving/disintegrating tablets (FDDTs) are known in the art of pharmaceutical development. Some of the more popular ones include effervescent dosage forms such as Alka-Seltzer® that are disintegrated in a disintegration media prior to administration.

[044] Other types of FDDTs include tablets that are formulated to dissolve or disintegrate in the mouth or buccal cavities. These orally-dissolving tablets (ODTs) may also be designed to have variable sub-lingual dissolution/disintegration times. For example, US Patent 7,282,217 discusses an ODT formulation that has a variable disintegration time of 2 to 120 seconds in the mouth, or R. P. Scherer Corp's fast dissolving drug delivery system, Zydis®, that has a total dissolution/disintegration time of 3 to 5 seconds. Eurand Corporation's AdvaTab™ technology provides another example, which combines taste-masking and controlled-release technologies.

[045] Other commercially available ODT technologies include GlaxoSmithKline's anti-nausea drug, Zofran®, manufactured by Cardinal Health Inc. and the OraSolv® and DuraSolv® systems developed and sold by Cima Labs, Inc. (Eden Prairie, MN). OraSolv® is an orally disintegrating tablet that combines taste masked active drug ingredients with a low effervescence system. DuraSolv® is a durable orally disintegrating tablet that combines taste masked active drug ingredients, and can be blistered-packed or bottled. In addition, OraVescent® is an enhanced-absorption drug delivery system intended to improve the transport of active drug ingredients across mucosal membranes. OraVescent® can be

administered buccally or sublingually. This technology may improve bioavailability and accelerate the onset of action of some active drug ingredients.

[046] Prior to the instant invention, these rapidly disintegrating formulations have never been used to be used to deliver protein therapeutics. It was not known whether the protein therapeutic would retain sufficient biological activity in these formulations. It is only with the results demonstrated in the Examples below that one of skill in the art would recognize that these formulations would be useful for the delivery of protein therapeutics such as liprotamase.

[047] The compositions of the invention all contain at least an active protein therapeutic and an effervescent agent. The effervescent agent may be an effervescent couple or it may be a single agent that is induced to effervesce in disintegration media or in the body. An effervescent couple is an acid and a carbonate salt, such as citric acid and sodium bicarbonate. An alternative acid is fumaric acid. An alternative salt is sodium carbonate. In some embodiments, the formulation contains the salt but not the acid, and the effervescence is induced by adding the formulation to an acidic disintegration media, such as juice.

[048] The compositions of the invention may contain some or all of the following excipients: binders, diluents, disintegrants, sweeteners, flavors, and lubricants. Binders hold the ingredients in a tablet together.

[049] Binders ensure that tablets and granules can be formed with required mechanical strength, and give volume to low active dosis tablets. Binders are usually starches, sugars, cellulose or modified cellulose such as microcrystalline cellulose, hydroxypropyl cellulose, lactose, cellulose, methyl cellulose, polyvinylpyrrolidone, and polyethylene glycol, or sugar alcohols like xylitol, sorbitol or maltitol,.

[050] Diluents fill out the size of a tablet, making it practical to produce and convenient for the consumer to use. By increasing the bulk volume, the diluents make it possible for the final product to have the proper volume for patient handling. A good diluent must be inert, compatible with the other components of the formulation, non-hygroscopic, soluble, relatively cheap, compactible, and preferably tasteless or pleasant tasting. Exemplary diluents include plant cellulose, dibasic calcium phosphate, lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate, and magnesium stearate.

[051] Disintegrants expand and dissolve when wet causing the tablet to break apart in the digestive tract, releasing the active ingredients for absorption. They ensure that when the tablet is in contact with water, it rapidly breaks down into smaller fragments, thereby facilitating dissolution. Examples of disintegrants include: crosslinked polyvinyl pyrrolidone, sodium starch glycolate, crosslinked sodium carboxymethyl cellulose (crosscarmellose), and crospovidone.

[052] Lubricants prevent ingredients from clumping together and from sticking to the tablet punches or capsule filling machine. Lubricants also ensure that tablet formation and ejection can occur with low friction between the solid and die wall. Exemplary lubricants include common minerals like talc or silica, and fats, such as vegetable stearin, magnesium stearate or stearic acid.

[053] Sweeteners are added to make the ingredients more palatable, especially in chewable or dissolvable tablets. Exemplary sweeteners include, sucrose, fructose, sucralose, and aspartame.

[054] Flavors can be used to mask unpleasant tasting active ingredients and improve the likelihood that the patient will complete a course of medication. Flavorings may be natural (e.g. fruit extract) or artificial. For example, to improve: a

bitter product, mint, cherry or anise may be used. Exemplary flavors include cherry berry, orange, mint, grape, and cherry.

[055] Additional examples of excipients are described in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and the Pharmaceutical Society of Great Britain. With respect to the compositions, according to this invention, excipients are inactive ingredients, and lipase, protease and amylase are active ingredients. The ratio of active to inactive ingredients in the compositions of this invention, on a w/w basis, may between about 1:24 to about 24:1; preferably from about 1:9 to about 9:1, most preferably between about 1:6 to about 6:1.

[056] In exemplary embodiments, the formulations of the invention comprise the following ingredients:

Table 1

COMPONENT	RANGE % w/w	PREFERRED % w/w
Active Ingredient: liprotamase	4.0 to 50.0	5.0 to 25.0
Diluent: Mannitol; Sorbitol	30 to 90	60 to 75
Binder: Microcrystalline Cellulose (MCC)	2.0 to 20.0	4.0 to 8.0
Disintegrant: Crospovidone	2.0 to 20.0	4.0 to 8.0
Effervescent Couple: Sodium Bicarbonate & Citric Acid	2.0 to 30.0	4.0 to 15.0
Sweetener: sucralose	0.20 to 4.0	0.50 to 2.0
Flavor: cherry berry	0.25 to 4.0	0.50 to 2.0
Lubricant: Magnesium Stearate; Stearic Acid	0.5 to 15.0	1.0 to 4.0
Total	**100**	**100.00**

[057] In exemplary embodiments, the compositions of the invention comprise the following properties:

Table 2

PARAMETER	PREFERRED RANGE	TARGET RANGE
Lipase CLEC Activity	5,000 - 65,000 Units	8,000 -32,500 Units
Protease SAB Activity	5,000 – 50,000 Units	6,000 – 50,000 Units

Amylase BZF Activity	500 - 7,500 Units	500 - 3,750 Units
Tablet Size	1/4 to 3/4 inch diameter	5/16 to 9/16 inch diameter
Tablet Weight	100 to 1200 mg	200 to 500 mg
Disintegration Time	15 secs to 3 minutes	30 sec; 1 min or less in applesauce
Packaging	Foil; Foil Blisters	
Flavors/	Fruit; mint; cherry; berry; bubblegum	
Color	White	

[058] A major aspect of the invention contemplates methods and techniques for selecting the appropriate disintegration media for disintegrating and dispersing the aforementioned oral dosage forms so that the protein therapeutic maintains all or some of its therapeutic activity before, during, and after the disintegration and also throughout the entire or partial administration of the disintegrated and dispersed products and media.

[059] The choice of disintegration media is very important for delivery and function of active ingredients in the composition. Because uncrystallized lipase is quickly degraded by protease, it is important that the lipase remain in crystallized form, which is impervious to protease activity, through the stomach, until it reaches the area in which it is intended to be functional – the (duodenum, jejunum, ileum). Thus, cross-linked crystallized forms of lipase are preferred because the crystals remain undissolved at gastric pH levels of (2.8 to 4.0). The crystallized lipase is impervious to protease activity. At higher pH levels typical of the lower digestive tract – i.e., the duodenum and the small intestine, the lipase crystals become solubilized and can act.

[060] In one aspect of the invention, the disintegration media may comprise of one or more solids, semi-solids, liquids, or combinations thereof (i.e.,

juices (e.g., apple juice) , milk, yogurt, puddings (e.g., rice pudding), cereal, carbonated beverages, etc.) that are possible for consumption.

[061] According to a preferred embodiment, the compositions of this invention are useful in methods for treating pancreatic insufficiency in any subject, including those suffering from cystic fibrosis. According to an alternative embodiment, the compositions of this invention are useful in methods for treating malabsorption in a subject. The compositions of the invention may be used to improve the nutritional status of a subject as measured by weight, height, and/or BMI of the subject. Further embodiments of this invention include use of the compositions of this invention for increasing the coefficient of fat absorption, or for increasing the coefficient of nitrogen absorption in a subject. Another embodiment of this invention includes use of those compositions to increase both the coefficient of fat absorption and the coefficient of nitrogen absorption in a subject, optionally by the same amount. In a further embodiment, the compositions of this invention are useful in methods for increasing carbohydrate absorption in a subject.

[062] In some embodiments, the patients treated with the compositions of the invention are useful for the treatment of patients with the inability to swallow capsules (infants and children), patients with difficulty swallowing capsules (older children, adolescents, and the elderly), and patients on GI tubes.

[063] The methods for treatment using the compositions according to this invention comprise the step of administering to a subject a therapeutically effective amount of such a composition. Any of the methods of this invention may be used to treat any subject suffering from pancreatic insufficiency, including cystic fibrosis patients.

EXAMPLES

Example 1--Formulation of rapidly disintegration tablets

[064] The following ingredients (Table 3) were weighed individually and screened through a 20 mesh hand sieve. These ingredients, except for the magnesium stearate, were then added to a V blender where they were mixed for 30 minutes. The magnesium stearate was then added to the blended mixture and mixing continued for an additional 5 minutes. The composition was then formed into tablets by compression using conventional rotary tableting equipment.

Table 3

Ingredient	mg/tablet	% w/w
Active ingredient--liprotamase		
Lipase	**6.68**	**2.23**
Protease	**9.54**	**3.18**
Amylase	**18.75**	**6.25**
Diluent		
mannitol	**208.52**	**69.51**
Binder		
microcrystalline cellulose	**15.00**	**5.00**
Disintegrant		
Crospovidone	**15.00**	**5.00**
Effervescent Couple		
Sodium bicarbonate	**8.70**	**2.90**
Citric acid	**6.30**	**2.10**
Sweetener		
Sucralose	**3.00**	**1.00**
Flavor		
cherry berry	2.50	0.83
Lubricant		
Magnesium Stearate	**6.00**	**2.00**
Total	**300**	**100**

[065] These tablets were tested for tablet weight variation, hardness, acitivity, uniformity, moisture content, and disintegration times as shown in Table 4.

Table 4

Properties	Target	Actual
Mean weight	300 mg	301 mg
Mean weight range	285-315 mg	291-218 mg
Mean hardness	13 N	11 N
Mean hardness range	10-16 N	7-19 N
Moisture content		0.9%
Disintegration time range	30	15-29 seconds
Activity (% LC)		
lipase		120.1
protease		104.1
amylase		144.6
Mean content uniformity (%RSD)		
lipase		119.0
protease		98.9
amylase		145.2

Example 2--Solubility studies

[066] Cross-linked lipase crystals were insoluble in apple cranberry juice (pH 2.8); citrate buffer (pH 6.0); and milk (pH 6.8). Cross-linked lipase crystals had limited solubility in Simulated Intestinal Fluid (SIF; 200 mM KCl; pH 7.5); and was readily soluble in Tris (pH 8.5). Protease was immediately soluble in all dissolution media. Amylase protein was readily soluble in all dissolution media.

[067] When added to the disintegration media in combination, cross-linked lipase crystals were observed in apple cranberry juice and milk, while the protease and amylase proteins were immediately dissolved. In Tris, lipase CLECs were visibly dissolved, and the Protease and Amylase proteins had immediately dissolved, too.

Example 3--Disintegration times

[068] Tablet disintegration was tested in a variety of media and the time of tablet disintegration was measured and is shown in Table 5.

Table 5 Tablet Disintegration Properties

Media	V, mL	pH Pre	pH, Post	Disintegration Time
Dis H_2O	50	5.0	5.6	24"
Dis H_2O	5	5.0	5.9	34"
5% Glycerol	5	4.8	6.1	42"
Apple Juice	5	3.6	4.2	1'
Apple Sauce	5	3.2	4.2	5'
Sweet Potato	5	5.3	5.6	7'
Peas Puree	5	6.1	6.0	3'

Example 4--Stability studies

[069] Table 6 reports the T=0 to 6-month stability of a composition prepared as set forth in Example 1 stored at 30°C / 65% RH (relative humidity) and 40°C / 75% RH. The results are normalized to their respective values at a storage condition of 5°C, and expressed as the percentage of specific activity retained over time. The 5°C storage represents a condition at which all samples are theoretically expected to remain completely stable.

Table 6: Stability of Prototype Rapidly Disintegrating Tablet

30°C / 65% RH		40°C / 75% RH	
Time (Months)	% Label Claim	Time (Months)	% Label Claim
0.0	**100.0**	0.0	**100.0**
1.5	**98.8**	1.5	**100.1**
3.0	**102.3**	3.0	**102.7**
6.0	**97.1**	6.0	**95.0**

30°C / 65% RH		40°C / 75% RH	
Time (Months)	% Label Claim	Time (Months)	% Label Claim
0.0	100.0	0.0	100.0
1.5	102.8	1.5	100.8
3.0	99.8	3.0	95.6
6.0	90.6	6.0	92.6

30°C / 65% RH		40°C / 75% RH	
Time (Months)	% Label Claim	Time (Months)	% Label Claim
0.0	100.0	0.0	100.0
1.5	98.0	1.5	94.9
3.0	99.0	3.0	94.4
6.0	101.1	6.0	96.3

[070] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A composition comprising from about 4.0 to about 50.0 weight percent of a mixture of lipase, protease, and amylase; from about 30 to about 90 weight percent excipients; and from about 2.0 to about 30.0 weight percent effervescent agent, wherein the composition is capable of rapidly disintegrating in the oral cavity.

2. The composition of claim 1, wherein the said rapidly disintegrating dosage form is a tablet.

3. The composition of claim 1, wherein one or more of the lipase, the protease and the amylase of liprotamase are recombinantly produced.

4. The composition of claim 1, wherein the lipase is crystallized.

5. The composition of claim 1, wherein the crystallized lipase is cross-linked.

6. The composition of claim 1, wherein the lipase, protease and amylase are present in the ratio of about: 1.0:0.77:0.12 USP units of enzyme activity, respectively.

7. The composition of claim 1, wherein the lipase crystals remain undissolved at gastric pH levels of 2.8 to 4.0.

8. The composition of claim 1, wherein the composition disintegrates in water in about 30 seconds to 2 minutes.

9. The composition of claim 1, wherein the lipase, amylase, and protease maintain about 50-100% of their initial biological activity after disintegration in water.

10. The composition of claim 1, wherein the composition maintains at least 50% of the initial biological activity for at least 2 hours after disintegration in water.

11. The composition of claim 1, wherein the effervescent agent is an effervescent couple.

12. The composition of claim 11, wherein the effervescent couple is sodium bicarbonate and citric acid.

13. The composition of claim 1, wherein the effervescent agent is sodium bicarbonate.

14. The composition of claim 1, wherein the excipients comprise one or more of a lubricant, a sweetener, a disintegrant, a binder, and a diluent.

15. The composition of claim 14, wherein the lubricant is selected from the group consisting of magnesium stearate and stearic acid.

16. The composition of claim 14, wherein the sweetener is sucralose.

17. The composition of claim 14, wherein the disintegrant is crospovidone.

18. The composition of claim 14, wherein the diluent is selected from the group consisting of mannitol and sorbitol.

19. The composition of claim 14, wherein the binder is microcrystalline cellulose.

20. A method of providing a composition of lipase, protease, and amylase to a patient in need of same comprising combining the rapidly disintegrating dosage form of claim 1 with a suitable disintegration media.

21. The method of claim 20, wherein the disintegration media is cranberry apple juice, water, or apple juice.

22. A method for reducing gastric tube occlusions, the method comprising treating a gastric tube with the composition formed by the method of claim 20.

23. Use of a composition of any one of claims 1 to 19 in the preparation of a medicament for the treatment of pancreatic insufficiency.

24. A method of treating pancreatic insufficiency in a patient comprising administering a composition of any one of claims 1 to 19 to a patient in need of same.

25. The method of claim 24, wherein the patient is unable to swallow capsules, has difficulty swallowing capsules, or is on a GI tube.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/054866

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/46 A61K38/47 A61K38/48 A61K9/46 A61P1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 750 104 A (SIPOS TIBOR [US]) 12 May 1998 (1998-05-12) column 1, lines 9-19 column 5, line 62 - column 7, line 27 example 3 claims	1-19, 23-25
X	GB 1 107 824 A (MAUVERNAY ROLAND YVES) 27 March 1968 (1968-03-27) column 1, lines 14-44 claims	1-22
X	EP 0 093 784 A (THILO CONTACTLINSEN [DE]) 16 November 1983 (1983-11-16) page 4, line 18 - page 5, line 4 page 6, lines 8-18 claims	1-21

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 November 2009	18/11/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giró, Annalisa

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/054866

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 079 125 A (SIPOS TIBOR) 14 March 1978 (1978-03-14) column 3, lines 16-51 column 4, line 67 - column 5, line 45 examples 1,5,6,8 claims	1-25
Y	WO 2006/044529 A (ALTUS PHARMACEUTICALS INC [US]; MARGOLIN ALEXEY L [US]; SHENOY BHAMI C) 27 April 2006 (2006-04-27) paragraph [0016] paragraphs [0024], [0025] paragraph [0044] paragraphs [0049], [0050] paragraphs [0072], [0073] paragraph [0077] paragraphs [0082], [0083] claims	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/054866

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5750104 A	12-05-1998	CA 2228389 A1	30-07-1999
GB 1107824 A	27-03-1968	NONE	
EP 0093784 A	16-11-1983	AT 28757 T	15-08-1987
		DE 3276909 D1	10-09-1987
US 4079125 A	14-03-1978	AU 504584 B2	18-10-1979
		AU 1475576 A	15-12-1977
		BE 842822 A1	10-12-1976
		DE 2626109 A1	30-12-1976
		DK 256776 A	11-12-1976
		FI 761645 A	11-12-1976
		FR 2313916 A1	07-01-1977
		GB 1509866 A	04-05-1978
		IE 43601 B1	08-04-1981
		IN 144157 A1	01-04-1978
		JP 52003819 A	12-01-1977
		NL 7606288 A	14-12-1976
		NO 761981 A	13-12-1976
		SE 7606470 A	11-12-1976
WO 2006044529 A	27-04-2006	AU 2005295754 A1	27-04-2006
		BR PI0516515 A	16-09-2008
		CA 2584069 A1	27-04-2006
		CN 101068565 A	07-11-2007
		EP 1809320 A1	25-07-2007
		JP 2008516965 T	22-05-2008
		KR 20070074622 A	12-07-2007